## Phylogeny

MAP2K4 is assigned to the STE group, MAP2K (STE7) family of the human kinome (roskoski2012erk12mapkinases pages 2-4).  
It shares approximately 50 % amino-acid identity with the paralog MAP2K7 (katzengruber2023mkk4inhibitors—recentdevelopment pages 2-4).  
Verified orthologs include Mus musculus Map2k4, Danio rerio map2k4, Drosophila melanogaster hemipterous and Caenorhabditis elegans mek-1 (krishna2013afluorescencebasedthermal pages 1-3).

## Reaction Catalyzed

ATP + [substrate]-L-threonine → ADP + [substrate]-O-phospho-L-threonine (avruch2007mapkinasepathways pages 5-6)  
ATP + [substrate]-L-tyrosine → ADP + [substrate]-O-phospho-L-tyrosine (avruch2007mapkinasepathways pages 5-6)

## Cofactor Requirements

Catalytic activity requires divalent cations Mg²⁺ or Mn²⁺ (katzengruber2023mkk4inhibitors—recentdevelopment pages 4-6).

## Substrate Specificity

MAP2K4 phosphorylates the TXY activation loops of MAPK8/JNK1 (Thr183/Tyr185), MAPK9/JNK2, MAPK10/JNK3 and MAPK14/p38α (Thr180/Tyr182) (katzengruber2023mkk4inhibitors—recentdevelopment pages 2-4).  
Kinase-profiling places MAP2K4 among proline-directed Ser/Thr kinases that favour SP/TP motifs with basic residues at the −3 to −5 positions (raman2007differentialregulationand pages 12-13).  
Enzymology demonstrates higher catalytic efficiency toward the Tyr185 position of the JNK TPY motif than the corresponding Thr site (katzengruber2023mkk4inhibitors—recentdevelopment pages 2-4).

## Structure

The 399-residue protein comprises an N-terminal JNK-binding D-domain, a bilobal kinase domain (≈ residues 80–360) and a C-terminal DVD domain that mediates MAP3K docking (katzengruber2023mkk4inhibitors—recentdevelopment pages 2-4).  
Crystal structure PDB 3ALN shows a five-β-strand N-lobe and predominantly α-helical C-lobe flanking an ATP-binding cleft (katzengruber2023mkk4inhibitors—recentdevelopment pages 1-2).  
Key catalytic motifs include the glycine-rich loop, HRD catalytic triad and DFG motif; Cys246 immediately N-terminal to the DFG sequence forms the nucleophile targeted by covalent inhibitors (katzengruber2023mkk4inhibitors—recentdevelopment pages 2-4).  
The activation loop contains Ser257 and Thr261 within the S-X-A-K-T sequence and is flexible until dual phosphorylation (hudson2018truncationandmotifbased pages 5-6).  
Unphosphorylated MAP2K4 forms a symmetric dimer that dissociates upon activation-loop phosphorylation (katzengruber2023mkk4inhibitors—recentdevelopment pages 1-2).

## Regulation

Full activation requires phosphorylation of Ser257 and Thr261 by upstream MAP3Ks such as MEKK1, MLK, ASK1 and TAK1 (avruch2007mapkinasepathways pages 2-3).  
Poly-ubiquitination by the E3 ligase Itch targets MAP2K4 for proteasomal degradation (katzengruber2023mkk4inhibitors—recentdevelopment pages 16-18).  
Substrate-peptide engagement can stabilise an autoinhibited conformation, providing allosteric control (katzengruber2023mkk4inhibitors—recentdevelopment pages 16-18).

## Function

MAP2K4 is expressed ubiquitously in adult tissues and is enriched in central nervous system and liver during embryogenesis (katzengruber2023mkk4inhibitors—recentdevelopment pages 2-4).  
Upstream activators include ASK, MEKK1-4, MLK family members and TAK1 (avruch2007mapkinasepathways pages 2-3).  
Direct substrates are MAPK8/JNK1, MAPK9/JNK2, MAPK10/JNK3 and MAPK14/p38α (nakayama2012map2k4(mitogenactivatedprotein pages 1-3).  
The N-terminal D-site binds JNK1/2 with higher affinity than ERK2, conferring signalling specificity (ho2003adockingsite pages 9-9).  
MAP2K4 functions within stress-activated JNK and p38 MAPK cascades that regulate cell proliferation, differentiation and apoptosis (katzengruber2023mkk4inhibitors—recentdevelopment pages 1-2).

## Inhibitors

Electrophilic inhibitors that covalently modify Cys246 achieve nanomolar potency against MAP2K4/7 (katzengruber2023mkk4inhibitors—recentdevelopment pages 16-18).  
A 9-H-pyrimido[4,5-b]ind-6-ol scaffold inhibits MAP2K4 with IC₅₀ values below 1 µM (katzengruber2023mkk4inhibitors—recentdevelopment pages 4-6).  
Natural product 7,3′,4′-trihydroxyisoflavone blocks MAP2K4 activity at ~1 µM in UVB-responsive assays (katzengruber2023mkk4inhibitors—recentdevelopment pages 4-6).  
Genistein inhibits MAP2K4 with an IC₅₀ of about 0.4 µM and reduces metastatic prostate cancer cell invasion (krishna2013afluorescencebasedthermal pages 4-6).

## Other Comments

Missense variant G265D in the activation segment diminishes catalytic activity and appears in gastric cancer genomes (hudson2018truncationandmotifbased pages 5-6).  
Somatic loss-of-function mutations and deletions in colorectal, lung, melanoma and ovarian cancers cluster within the kinase domain (nakayama2012map2k4(mitogenactivatedprotein pages 1-3).  
Catalytic-domain mutation R134W has been reported in tumour sequencing studies (katzengruber2023mkk4inhibitors—recentdevelopment pages 16-18).  
Over-expression correlates with aggressive prostate, ovarian and triple-negative breast cancers (katzengruber2023mkk4inhibitors—recentdevelopment pages 1-2).

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